United States Environmental Protection Agency **Office of Research and Development**

National Exposure Research Laboratory
Research Abstract
Government Performance Results Act Goal: Clean and Safe Water

Significant Research Findings:

Methods for Detecting Arsenic Species in Food

Scientific Problem and Policy Issues

The maximum contaminant level (MCL) for arsenic in drinking water is currently being revised. The formulation of this MCL is influenced by a variety of factors including best available treatment technology, analytical monitoring capability, and risk assessments based on health data and relative exposure source estimates. The relative exposure source estimate is used to assess arsenic exposure from all possible routes. For arsenic, the two major exposure routes are dietary and drinking water ingestion. Arsenic dietary exposure data is needed to improve cancer risk estimates. Food, unlike drinking water, can contain numerous forms or species of arsenic which vary significantly in relative toxicity. Many foods are comprised of mostly less toxic forms of arsenic. However, one of the primary dietary sources of arsenic is seafood, and there is great uncertainty whether or not seafoods are a significant source of toxic forms of arsenic. In many cases, the predominant "extractable" arsenical associated with seafood is a low toxicity species, arsenobetaine. However, in certain seafood matrices, less than 50% of the arsenic can be extracted and identified. The remaining "unextractable" arsenic fraction in these foods can lead to considerable uncertainty in dietary arsenic risk estimates because the risk/toxicity of the unextractable arsenic is unknown. Other dietary samples, such as carrots and rice, have similar issues regarding species-specific determinations and extractability. Thus, the main objective of dietary arsenic exposure research focuses on developing analytical approaches that provide a complete or quantitative extraction of the arsenic prior to speciation analysis. This research by the U.S. EPA's National Exposure Research Laboratory (NERL) has involved separate collaborative efforts with both the U.S. Food and Drug Administration (FDA) and U.S. EPA Region 10.

Research Approach

Research has focused on minimizing the unextractable arsenic in dietary samples known to be high in arsenic. These samples were selected based on total arsenic analyses conducted for the FDA's market basket survey. Because most of the arsenic in a diet is associated with only a few foods, it is possible to obtain a fairly robust estimate of dietary arsenic by analyzing a target set of foods. For example, seafood represents greater than 60% of the total arsenic in the diet for the general U.S. population, but has poor extraction efficiencies. Rice is another food group which is known to be high in arsenic and is problematic in extracting and speciating arsenic.

The analytical approach initially used to extract the solid dietary samples was Accelerated Solvent Extraction (ASE). This extraction technique has the advantage of being a semi-automated approach which minimizes the overall cost of the analysis and provides procedural repeatability. However, while the ASE was found to produce quantitative extraction in certain seafoods, carrot and rice samples, other seafood (oysters, clams) and rice (long grain, brown) samples produced extraction efficiencies of less than 60%. The extraction step was followed by a separation via Ion Chromatography (IC). Arsenic species have either a net positive or negative charge depending on the pH used in the chromatographic separation. Positively charged arsenic are separated on a cation column, and the negatively charged species are separated on a anion column. Because seafoods contain a wide variety of arsenicals, both cation and anion separations are required to properly quantitate each individual species. Rice, carrots and apples contain fewer arsenicals, and for this reason an anion separation was found to be adequate. In all cases, Inductively Coupled Plasma Mass Spectrometry (ICP-MS) was used as the detector for the IC separation. In some early development work, Ion Chromatography Electrospray Ionization Mass Spectrometry/ Mass Spectrometry (IC-ESI-MS/MS) was used to structurally identify unknown arsenic compounds which eluted from the IC column.

Results and Implications

This Annual Performance Measure (APM 65) supports FY01 Annual Performance Goal 009 which states: "By 2001, reduce uncertainties and improve methods associated with the assessment and control of risks posed by exposure to arsenic in drinking water." Significant findings were as follows:

1.) ASE was evaluated as an extraction procedure by both the EPA and the FDA. ASE compared favorably to conventional extraction techniques, but, in certain seafoods, both ASE and conventional extraction failed to produce quantitative extractions (*JAAS,* 1999, 14, 607). Subsequent analysis of seaweed products by Gallagher (*FJAC*, 2001, 369,71) indicated that extraction efficiencies in seaweed can be as low as 25%. Similar results were obtained by the FDA for carrots (*Analyst,* submitted 3/01), rice (*JAAS*, 2001, 16, 299) and apples (*Analyst*, 2001, 126, 136). A 25% extraction efficiency implies that 75% of the arsenic remains in the sample undetected. For example,

seafood samples which can contain up to 10,000 ppb (μg/L) arsenic would contain 7,500 ppb of undetected and potentially toxic arsenic. This dietary source of arsenic may be significant when compared to the old drinking water MCL of 50 ppb, and the relative significance would increase with lower drinking water MCLs. Species-specific losses associated with the ASE sample container and dispersion media (used to improve contact time between the sample and extraction solvent) was also investigated (Gallagher, submitted 9/01) and were reduced by utilizing inert dispersion media. However, subsequent evaluations indicated that the ASE could not quantitatively extract samples such as oysters, clams and certain varieties of rice. These results clearly indicated that the risk associated with certain exposures would be underestimated using the ASE or conventional extractors.
2.) Arsenosugars, commonly associated with clams, oysters and seaweed products, produced false positives due to chromatographic co-elution with other toxic species present in the sample. Because arsenosugar standards are not available commercially, they were structurally identified and characterized by IC-ESI-MS/MS and IC-Hydride ICP-MS (*JAAS,* 1999,14, 607). This characterization allowed for the development of a chromatographic separation which fully separated the arsenosugars from other arsenic species. Because the arsenosugars can be as high as 5,000 ppb in certain seafoods, the misidentification of these as more toxic species can influence risk assessments associated with seafood ingestion. Although there is currently a large degree of uncertainty regarding the relative toxicity of arsenosugars, the elimination of this false positive is likely significant when viewed relative to a drinking water MCL of 50 ppb.

In summary, this research has resulted in a number of analytical insights for speciating arsenic in dietary samples. The use of IC-ICP-MS in combination with IC-ESI-MS/MS for the structural verification of arsenosugars in natural product extracts indicates that these sugars can produce false positives at concentration levels of 1,000 ppb (which greatly exceeds the old drinking water MCL of 50 ppb) (Gallagher *JAAS,* 1999,14, 607). A second analytical discovery is that the use of ASE as a semi-automated extraction device, as well as conventional extraction techniques, produce matrix dependent extraction efficiencies. Therefore, the risk associated with certain seafoods and rice samples may be highly uncertain given the inherently poor extraction efficiencies.

Research Collaboration and Publications

This research has been a collaborative effort between the U.S. EPA's National Exposure Research Laboratory and the U.S. Food and Drug Administration (FDA) and between NERL and U.S. EPA Region 10.

U.S. EPA Publications and Presentations:

Gallagher, P.A., Creed, J.T., Wei, X. Murray, S., Brockhoff, C.A. "An Evaluation of Sample Dispersion Medias with ASE for the Extraction and Recovery of Arsenicals in LFB and DORM-2 with ICP-MS Detection." *Analyst* submitted 9/01.

Gallagher, P.A., Gamble, B.M., Heck, A.N., Freeman, D.M., Schwegel, C.A., Creed, J.T. "Characterization of Arsenosugars and Associated Degradation Products Following and Aggressive Acid / Base Extraction Procedure" Presented at the International Symposium on Environmental Toxicology of Metals and Metalloids - Environmental Chemistry, Toxicology and Health, Queensland, Australia, July, 2001.

Gallagher, P.A., Creed, J.T., Wei, X., Shoemaker, J., Schwegel, C.A. "Extraction and Detection of Arsenicals in Seaweed via ASE with IC-ICP-MS Detection." Fresenius *Journal of Anal. Chem.* 369: 71-80, 2001.

Gamble, B.M., Gallagher, P.A., Heck, A.N., Schwegel, C.A., Creed, J.T. "An Investigation of the Chemical Stability of Arsenosugars in Extraction Solvents Utilized to Quantitatively Extract Arsenicals from Seafood Products using IC-ICP-MS Detection" Presented at the European Winter Conference on Plasma Spectrochemistry, Lillihamer, Norway, February, 2001.

Gallagher, P.A., Heck, A.N., Wei, X., Schwegel, C.A., Creed, J.T. "A Comparison of Extraction Efficiencies in Seafood Matrices using a Synthetic Stomach and an Accelerated Solvent Extraction approach with IC-ICP-MS Detection" Presented at the European Winter Conference on Plasma Spectrochemistry, Lillihamer, Norway, February, 2001.

Creed, J.T., Gallagher, P.A., Wei, X., Schwegel, C.A. "Extraction Techniques for the Removal of Arsenicals from Seafood Exposure Matrices with ICP-MS Detection" Presented at the Fourth International Symposium on Speciation of Elements in Biological, Environmental and Toxicological Sciences, British Columbia, Canada, July, 2000.

Creed, J.T., Gallagher, P.A., Wei, X., Schwegel, C.A., Larenzana, R., Chamberlain, I. "Accelerated Solvent Extraction of Arsenicals from Seafood Matrices with Ion Chromatography and ICP-MS Detection" Presented at the Fourth International Conference on Arsenic Exposure and Health Effects, San Diego, CA, June, 2000.

Gallagher, P.A., Wei, X., McKiernan, J.W., Schwegel, C.A., Murray, S., Creed, J.T. "Accelerated Solvent Extraction of Arsenicals from Environmental Matrices with Ion Chromatography Separation and ICP-MS Detection" Winter Conference on Plasma Spectrochemistry, Ft. Lauderdale, FL, January, 2000.

Wei, X., Shoemaker, J.A., Gallagher, P.A., Schwegel, C.A., Creed, J.T. "Extraction and Identification of Arsenosugars in Commercially Available Seaweeds" Presented at the International Ion Chromatogarphy Symposium, San Jose, CA, September, 1999.

Gallagher, P.A., Creed, J.T. Wei, X., Shoemaker, J., Brockhoff, C.A. "Detection of Arsenosugars from Kelp Extracts via IC-ESI-MS/MS and IC Membrane Hydride Generation ICP-MS." *Journal of Analytical Atomic Spectrometry* 14: 1829-1834, 1999.

McKeirnan, J., Brockhoff, C.A., Creed, J.T., Caruso, J. "A Comparison of Automated and Traditional Methods for the Extraction of Arsenicals from Fish." *Journal of Analytical Atomic Spectrometry* 14: 607-613, 1999.

Gallagher, P.A., Creed, J.T., Brockhoff, C.A., Wei, X., McKiernan, J.W., Caruso, J., Shoemaker, J. "Accelerated Solvent Extraction of Arsenicals in Seaweed with Ion Chromatographic Separation and ICP-MS Detection" Presented at the American Chemical Society, Anaheim, CA, March, 1999.

Gallagher, P.A., Creed, J.T., Brockhoff, C.A., Wei, X., McKiernan, J.W. "The Extraction and Detection of Arsenicals in Seaweed via Accelerated Solvent Extraction with Ion Chromatography Separation and ICP-MS Detection" Presented at the European Winter Conference on Plasma Spectrochemistry, Pau, France, January, 1999.

McKiernan, J.W., Creed, J.T., Brockhoff, C.A., Chamberlain, I. "An Evaluation of Accelerated Solvent Extraction as a Semi-Automated Means of Extracting Arsenicals Prior to Speciation Analysis via IC-ICP-MS" Presented at the European Winter Conference on Plasma Spectrochemistry, Pau, France, January, 1999.

U.S. FDA Publications and Presentations:

Vela, N.P., Heitkemper, D.T. "Arsenic Extraction and Speciation in Carrots using Accelerated Solvent Extraction, Ion Chromatography and Plasma Mass Spectrometry." *Analyst* 126: 1011-1017, 2001.

Heitkemper, D.T., Vela, N.P., Stewart, K.R., Westphal, C. "Determination of Total and Speciated Arsenic in Rice by Ion Chromatography and Inductively Coupled Plasma Mass Spectrometry." *Journal of Analytical Atomic Spectrometry* 16: 299- 306, 2001.

Heitkemper, D.T., B'hymer, C.B., Caruso, J.A. "Evaluation of Extraction Techniques for Arsenic Species from Freeze Dried Apple Samples." *Analyst* 126: 136-140, 2001.

Heitkemper, D.T., Vela, N.P., Creed, J.T., Wei, X., Shoemaker, J., Gallagher, P.A., Schwegel, C.A. "Investigation of Methylated Arsenic Species in Carrots" Presented at the Fourth International Conference on Arsenic Exposure and Health Effects, San Diego, CA, June, 2000.

Future Research

The poor extraction efficiencies mentioned above have led to the development of a tetramethylammonium hydroxide (TMAOH) extraction procedure for seafoods (generally high in protein) and a trifluoroacetic acid (TFA) extraction procedure for rice (high in starch). TMAOH "softens" the protein in seafoods, and TFA "softens" the starch in rice allowing more of the arsenicals to be released and speciated. This release means that the extraction procedures have removed almost all of the arsenic present in the sample. The extract is then injected onto a chromatographic column. Future research will address the inability to elute certain species from the chromatographic column. The inability to elute all species from a column is correlated to the presence of proteins in the extract. The unchromatographable species may be bound to a protein or other substrate which may require the addition of reagents which denature the protein and liberate the bound arsenicals. The minimization of this unwanted binding to substrates within the extract is essential to developing fully quantitative arsenic speciation methodologies.

Contacts for Additional Information

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